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(54) Title: METHOD FOR OBTAINING PRODUCTS ENRICHED IN PHOSPHO- AND SPHINGOLIPIDS

(57) Abstract: The invention relates to a method for obtaining products enriched in phospho- and sphingolipids, whereby these products are obtained by ultrafiltration over a membrane with a cut-off value preferably ranging from 5,000 to 20,000 Dalton. The product is obtained by ultrafiltration of by-products rich in water, directly obtained from the processing of milk or obtained from the further processing of these directly acquired by-products. The invention further relates to the use of a product enriched in sphingo- and phospholipids, obtained by ultrafiltration over a membrane, as basic material for processing into "functional food" or as basis for processing into "nutraceuticals" and also relates to food or a food supplement with an enriched sphingo- and phospholipid concentration, obtained by ultrafiltration over a membrane.

METHOD FOR OBTAINING PRODUCTS ENRICHED IN PHOSPHO- AND SPHINGOLIPIDS

The invention relates to a method for obtaining products enriched in phospho- and sphingolipids.

The importance of the role, which phospho- and sphingolipids play in the human body, is becoming clearer and clearer for the biochemical and the biomedical sector. Especially the group of sphingolipids is one of the most important groups of the lipids that are found in cell membranes. They intermediate in communication between cells, signal transduction, immunorecognition and the definition of the physical condition of membranes and lipoproteins. Moreover it has appeared from present research that sphingolipids can act as intracellular Ca^{2+} transmitters.

The importance of providing a simple industrial method for obtaining products enriched in phospho- and sphingolipids, which can be processed in the food industry, also immediately becomes evident because of this.

The purpose of the invention is therefore to obtain in a simple manner products enriched in phospho- and sphingolipids, therefore through the use of simple mechanical means and simple additives.

This purpose is achieved through a method for obtaining products enriched in phospho- and sphingolipids, whereby

the product is obtained by ultrafiltration over a membrane. The membrane preferably has a cut-off value below 20,000 Dalton.

Although generally phospho- and sphingolipids have a molecular weight that ranges from 300 to 1,000 Dalton, it is surprising to ascertain that the greatest portion of these phospho- and sphingolipids after ultrafiltration with membranes with cut-off values ranging from 3,000 and 20,000 Dalton are in the retentate. This phenomenon can be explained through the fact that the phospho- and sphingolipids are most probably still in fragments of natural fatty globule membranes, through which the phospho- and sphingolipids are retained.

In a preferred method the product is obtained by ultrafiltration of by-products rich in water, directly obtained from the processing of milk or obtained from the further processing of these directly acquired by-products.

This has the advantage that economically inferior products or waste products from the processing of milk can still be upgraded to an economically worthy and nutritional product.

In a more specific preferred method the aforementioned membrane has a cut-off value ranging from 5,000 to 10,000 Dalton.

This has the advantage that after ultrafiltration the phospho- and sphingolipids are found in an optimum amount in the retentate. Below the lower limit of 5,000 Dalton there is greater chance of membrane blockage and above the upper limit of 10,000 Dalton the phospho- and sphingolipids partly or fully permeate through the membranes.

In a preferred method the aforementioned by-products are casein-free prior to ultrafiltration.

The advantage thereof is that the casein can therefore have no negative effect on the product as ingredient, because casein is a dominant protein.

If the products are not yet casein-free, the aforementioned by-products are preferably made casein-free.

Through the implementation of the method a product is obtained that can serve as ingredient for "functional food", this is food with a health promoting affect or as basis for processing into "nutraceuticals", these are foodstuffs that clearly resemble a medicine.

In the method the following steps are preferably implemented:

- filter the casein-free by-product via cake filtration;
- adjust pH of the casein-free by-product with alkali to a pH between 6 and 7;

- separate the casein-free by-product by ultrafiltration into a retentate and a filtrate, whereby approximately all phospholipids, of which the sphingolipids are a part, are in the retentate.

This method has the advantage that it is simple and that simple means and simple additives are used.

The invention further relates to the use of a product enriched in sphingo- and phospholipids, obtained by ultrafiltration over a membrane, as basic material for the preparation of "functional food" or as basis for processing into "nutraceuticals".

The invention also relates to food or a food supplement that has an enriched sphingo- and phospholipid concentration, obtained by ultrafiltration over a membrane.

In order to obtain a product with a certain amount of phospho- and sphingolipids, all types of shunt flows are taken that originate from the processing of milk. There are processing possibilities of milk, among others:

- the skimming of milk, whereby cream is obtained. On the one hand butter can be made from the cream, with buttermilk as by-product. From the buttermilk casein can be extracted, through which whey occurs, which is a further by-product. From buttermilk fresh cheese of the quark type can further also be made (see PCT patent application PCT/EP/007963), whereby again whey occurs as by-product. The cream can also be processed

into butter oil, whereby apart from the butter oil cream serum occurs as by-product. Butter can also be processed into butter oil, whereby apart from the butter oil butter serum occurs as by-product.

- production of fresh and ripened cheeses, whereby apart from the cheese whey is obtained as by-product.

Out of all the by-products mentioned a product can be made that is enriched in phospho- and sphingolipids.

The by-products that are obtained with the preparation of butter and butter oil and with the further processing of these acquired by-products do give a greater output of sphingo- and phospholipids and are more economically processable.

If the by-products for the process are not casein-free the casein is removed prior to ultrafiltration. This can be effected on the one hand by acidification of the products containing casein by either lowering the pH with inorganic and organic acids to a pH of ± 4.6 (isoelectric point of casein) or adding lactic acid bacteria or on the other hand by rennet coagulation (= addition of the chymosin enzyme) of the products containing casein. The by-product is then centrifuged, through which a top casein-free fraction and a bottom casein fraction is obtained.

Then the final ultrafiltration process starts. The by-products that are already casein-free of the by-products made casein-free are filtered via cake filtration, for

example on a Buchner filter. The pH of the by-products is neutralised with alkali to a pH of ± 6.75. By ultrafiltration on a membrane with a cut-off value ranging from 5,000 to 10,000 Dalton, a retentate and a filtrate are obtained. Approximately all phospho- and sphingolipids are in the retentate.

Apart from the presence of the phospho- and the sphingolipids in the retentate, all other functional proteins, among which membrane proteins, immunoglobulins, lactoglobulins, etc. are also held in the retentate, because a membrane is used with a cut-off value below 20,000 Dalton.

Thus a concentrate is obtained that has an interesting nutritional value.

This concentrate can be further lyophilised or spray-dried, so that it is available in powder form. This powder can be further processed into "functional food" or into "nutraceuticals", for example in capsules or in the form of tablets.

The membranes that are used are organic membranes, among which pES (polyethylsulfone) membranes or cellulose membranes. Ceramic membranes can also be used, but these do give more sedimentary deposit of protein components and are therefore more economically disadvantageous.

The method according to the invention is illustrated in detail in the following examples.

Example 1

Preparation of a product enriched in phospho- and sphingolipids on the basis of sour buttermilk, obtained from the preparation of butter on the basis of acidulated cream, via ultrafiltration with a pES membrane with a cut-off value of 5000 Dalton.

Following process is implemented:

- acidulate 2000 ml buttermilk (pH 4.3) with HCl to pH 3.3;
- centrifuge 15 minutes at 3250 rpm;
- separate top whey fraction and bottom casein fraction;
- filter whey fraction on a Buchner filter with a Schleicher & Schuell 595 ½ filter paper;
- adjust pH of the whey fraction with NaOH to pH 6.75; the whey fraction has a volume of 500 ml;
- ultrafilter whey fraction, 390 ml retentate 1 and a 110 ml filtrate 1 are obtained;
- as test to see whether a further concentration is possible, the whey will again be ultrafiltered and 200 ml retentate 2 and 80 ml filtrate 2 are obtained.

The acquired fractions are analysed: the dry matter content, the amount of protein and the amount of fat can be seen in table 1; the amount of phospholipids and the amount of sphingomyelin can be seen in table 2.

Table 1:

	dry matter content (%)	amount of protein (%)	amount of fat (%)
buttermilk	9.45	3.66	0.72
whey fraction	6.27	0.65	0.37
casein fraction	13.15	7.83	1.13
retentate 1	6.54	0.74	0.33
filtrate 1	4.65	0.24	0.01
retentate 2	7.09	0.88	0.41
filtrate 2	5.00	0.27	0.01

Table 2:

	phospholipid content g PL/100 g product g PL/100 g dry matter	sphingomyelin content g SPM/g fat g SPM/100 g product g SPM/100 g dry matter
buttermilk	0.14 1.43	0.04 0.03 0.31
whey fraction	0.07 1.11	0.03 0.01 0.19
casein fraction	0.22 1.78	
retentate 1	0.13 1.95	
filtrate 1	-	
retentate 2	0.14 2.00	0.10 0.04 0.59
filtrate 2	-	

Example 2

Preparation of a product enriched in phospho- and sphingolipids on the basis of whey that occurs as by-product from the preparation of fresh low-fat cheese of the quark type (see PCT patent application PCT/EP/007963) by ultrafiltration with a pES membrane with a cut-off value of 8000 Dalton.

In this ultrafiltration process ultrafiltration is effected only once, through which a retentate and a filtrate are obtained.

The acquired fractions are analysed: the dry matter content and the amount of fat can be seen in table 3; the amount of phospholipids and the amount of sphingomyelin can be seen in table 4.

Table 3:

	dry matter content (%)	amount of fat (%)
whey	5.71	0.40
retentate	7.11	1.04
filtrate	4.60	0.03

Table 4:

	phospholipid content g PL/100 g product g PL/100 g dry matter	sphingomyelin content g SPM/g fat g SPM/100 g product g SPM/100 g dry matter
whey	0.07	0.02
	1.15	0.01
		0.15
retentate	0.20	0.02
	2.84	0.02
		0.26
filtrate	0.01	-
	0.10	

From the results of the analysis of the sphingomyelin content (g SPM/100 g dry matter), as shown in table 2 and table 4, it can be deduced that the sphingomyelin content of the retentate is double that of the sphingomyelin content of the whey.

It should be noted that this final concentration can still be increased by diafiltration, whereby water or a watery fraction is added to the retentate, after which concentration is again effected.

C L A I M S

1. Method for obtaining a product enriched in phospho- and sphingolipids **characterised in that** the product is obtained by ultrafiltration over a membrane.

2. Method according to claim 1, **characterised in that** the product is obtained by ultrafiltration of by-products rich in water, directly obtained from the processing of milk or obtained from the further processing of these directly acquired by-products.

3. Method according to one of the claims 1 and 2, **characterised in that** the aforementioned membrane has a cut-off value ranging from 5,000 to 10,000 Dalton.

4. Method according to one of the claims 1 up to and including 3, **characterised in that** the aforementioned by-products are casein-free prior to ultrafiltration.

5. Method according to one of the claims 1 up to and including 3, **characterised in that** the aforementioned by-products are made casein-free prior to ultrafiltration.

6. Method according to one of the preceding claims, **characterised in that** the following steps are implemented:

- filter the casein-free by-product via cake filtration;
- adjust pH of the casein-free by-product with alkali to a pH between 6 and 7;
- separate the casein-free by-product by ultrafiltration into a retentate and a filtrate, whereby approximately all phospho- and sphingolipids are in the retentate.

7. Use of a product enriched in phospho- and sphingolipids, obtained by ultrafiltration over a membrane, as basic material for the preparation of "functional food" or as basis for processing into "nutraceuticals".

8. Food or food supplement **characterised in that** these have an enriched sphingo- and phospholipid concentration, obtained by a method according to claims 1 up to and including 7.

INTERNATIONAL SEARCH REPORT

national Application No
PCT/BE 00/00130

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23C9/142 A23J7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23C A23J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, WPI Data, FSTA, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	PATENT ABSTRACTS OF JAPAN vol. 018, no. 086 (C-1165), 14 February 1994 (1994-02-14) & JP 05 292880 A (SNOW BRAND MILK PROD CO LTD), 9 November 1993 (1993-11-09) abstract --- -/--	1-5,7,8

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
8 August 2001	22/08/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-3016	Desmedt, G

Form PCT/ISA/210 (second sheet) (July 1992)

national Application No
PCT/BE 00/00130

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

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